

Attorney Docket No.: 78687-128 (RU-0238)
Inventors: Howell and Vorsa
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This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (previously presented): A substantially purified plant proanthocyanidin extract substantially free of anthocyanins and flavonols, wherein said extract is capable of inhibiting agglutination of P-type E. coli and not capable of inhibiting agglutination of type 1 E. coli.

Claim 2 (previously presented): The extract of claim 1, wherein said extract is substantially free of hydrolyzable tannins, alkaloids, lipids, carbohydrates, simple sugars, protein and amino acids, alcohols and organic acids.

Claim 3 (original): The extract of claim 1, wherein said plant extract is from a plant in the family Ericaceae, Rosaceae, Pinaceae or Vitaceae.

Claim 4 (original): The extract of claim 3, wherein said plant is from the family Ericaceae.

Claim 5 (original): The extract of claim 4, wherein said plant is a *Vaccinium* species.

Claim 6 (original): The extract of claim 5, wherein said *Vaccinium* species is selected from the group consisting of *Vaccinium macrocarpon*, *Vaccinium vitis-idaea*, *Vaccinium*

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oxycoccus, *Vaccinium augustifolium*, *Vaccinium ashei*, *Vaccinium corymbosum*, *Vaccinium lamarckii* and *Vaccinium myrtillus*.

Claim 7 (original): The extract of claim 5, wherein said *Vaccinium* species is *Vaccinium macrocarpon*.

Claim 8 (original): The extract of claim 3, wherein said plant is from the family Vitaceae.

Claim 9 (original): The extract of claim 8, wherein said plant is a *Vitis* species.

Claim 10 (original): The extract of claim 9, wherein said *Vitis* species is selected from the group consisting of *Vitis labrusca*, *Vitis rotundifolia* and *Vitis vinifera*.

Claim 11 (original): The extract of claim 7, wherein said extract comprises one or more proanthocyanidin compounds comprising two or more flavanoid monomer units wherein at least two of said units are linked together by an A-type interflavanoid linkage by bonds between C4 and C8 and between the C2 and the oxygen of C7 of the units and the remainder of any units are linked to each other by a B-type interflavanoid bond between C4 and C8 or between C4 and C6 of the units.

Claim 12 (original): The extract of claim 11, wherein said compounds consist of an average of from at least four to about seven epicatechin flavanoid monomer units.

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Claim 13 (original): The extract of claim 12, wherein said compounds consist of an average of four, five or six epicatechin flavanoid units.

Claims 14-19 (canceled).

Claim 20 (original): A method of preparing a proanthocyanidin extract from a plant which comprises:

(a) homogenizing plant material in an aqueous extraction solvent comprising at least about 10% water but no more than about 30% water, about -10% to about 70% acetone, about 5% to about 60% methanol and about 0.05% to about 1% ascorbic acid to prepare a first extract;

(b) subjecting said first extract to further purification;

(c) recovering a substantially purified proanthocyanidin extract, wherein

(d) said extract is capable of inhibiting agglutination of P-type *E. coli* and not capable

(e) of inhibiting agglutination of type 1 *E. coli*.

Claim 21 (original): The method of claim 20, wherein said plant material is from a plant in the family Ericaceae, Rosaceae, Pinaceae or Vitaceae.

Claim 22 (original): The method of claim 21, wherein said plant material is from a plant in the family Ericaceae.

Claim 23 (original): The method of claim 22, wherein said plant is a *Vaccinium* species.

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Claim 24 (original): The method of claim 23, wherein said *Vaccinium* species is selected from the group consisting of *Vaccinium macrocarpon*, *Vaccinium vitis-idaea*, *Vaccinium oxycoccus*, *Vaccinium augustifolium*, *Vaccinium ashei*, *Vaccinium corymbosum*, *Vaccinium lamarckii* and *Vaccinium myrtillus*.

Claim 25 (original): The method of claim 23, wherein said *Vaccinium* species is *Vaccinium macrocarpon*.

Claim 26 (original): The method of claim 21, wherein said plant is from the family Vitaceae.

Claim 27 (original): The method of claim 26 wherein said plant is a *Vitis* species.

Claim 28 (original): The method of claim 27, wherein said *Vitis* species is selected from the group consisting of *Vitis labrusca*, *Vitis rotundifolia* and *Vitis vinifera*.

Claim 29 (original): The method of claim 20, wherein said plant material is from leaves, mature fruit, immature fruit, stems or roots.

Claim 30 (original): The method of claim 29, wherein said plant material is from leaves.

Claim 31 (original): The method of any one of claims 20-30, wherein said aqueous extraction solvent comprises about 40% acetone, about 40% methanol and about 0.1% ascorbic acid.

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Claim 32 (original): In a method of isolating proanthocyanidins from plant material which comprises homogenizing said plant material with an extraction solvent to obtain a first extract, and subjecting said first extract to further purification steps to obtain a proanthocyanidin extract, the improvement which comprises homogenizing said plant material in an aqueous extraction solvent comprising at least about 10% water but no more than about 30% water, about 10% to about 70% acetone, about 5% to about 60% methanol and about 0.05% to about 0.2% ascorbic acid to obtain said first extract.

Claim 33 (original): The method of claim 32, wherein said plant material is from a plant in the family Ericaceae, Rosaceae, Pinaceae or Vitaceae.

Claim 34 (original): The method of claim 33, wherein said plant material is from a plant in the family Ericaceae.

Claim 35 (original): The method of claim 34, wherein said plant is a *Vaccinium* species.

Claim 36 (original): The method of claim 35, wherein said *Vaccinium* species is selected from the group consisting of *Vaccinium macrocarpon*, *Vaccinium vitis-idaea*, *Vaccinium oxycoccus*, *Vaccinium augustifolium*, *Vaccinium ashei*, *Vaccinium corymbosum*, *Vaccinium lamarckii* and *Vaccinium myrtillus*.

Claim 37 (original): The method of claim 35, wherein said *Vaccinium* species is *Vaccinium macrocarpon*.

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Claim 38 (original): The method of claim 33, wherein said plant is from the family Vitaceae.

Claim 39 (original): The method of claim 38, wherein said plant is a *Vitis* species.

Claim 40 (original): The method of claim 39, wherein said *Vitis* species is selected from the group consisting of *Vitis labrusca*, *Vitis rotundifolia* and *Vitis vinifera*.

Claim 41 (original): The method of any one of claims 32-40, wherein said aqueous extraction solvent comprises about 40% acetone, about 40% methanol and about 0.1% ascorbic acid.

Claim 42 (original): A method of preparing a proanthocyanidin extract from a *Vaccinium* species which comprises:

(a) homogenizing *Vaccinium* plant material in an aqueous extraction solvent comprising at least about 10% water but no more than about 30% water, about 10% to about 70% acetone, about 5% to about 60% methanol and about 0.05% to about 0.2% ascorbic acid to prepare a first extract;

(b) clarifying said first extract and obtaining a supernatant fraction therefrom;

(c) removing solvent from said supernatant fraction to obtain a residue and suspending said residue in distilled water to obtain an aqueous residue solution;

(d) subjecting said aqueous residue solution to further purification by either

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(i) applying said aqueous residue solution to reverse-phase lipophilic chromatography material equilibrated in distilled water and successively washing said lipophilic chromatography material with a sufficient amount of distilled water to remove sugars, a sufficient amount of about 15% aqueous methanol to remove acids and a sufficient amount of 100% acidified methanol to elute polyphenolic compounds, and then removing solvent from said polyphenolic compounds to obtain a first dried fraction, or

(ii) extracting said aqueous residue solution with a non-polar extraction solvent, recovering the aqueous phase thereof and removing solvent from said aqueous phase to obtain a second dried fraction; (e) suspending said first or second dried fraction in about 50% aqueous ethanol to obtain an ethanol solution, applying said ethanol solution to mixed hydrophilic-lipophilic chromatography material equilibrated in about 50% aqueous ethanol, and washing said mixed hydrophilic-lipophilic chromatography material with an amount of about 50% aqueous ethanol sufficient to remove non-proanthocyanidin polyphenolic compounds; and

(f) eluting said hydrophilic-lipophilic chromatography material with an amount of about 70% aqueous acetone sufficient to obtain said proanthocyanidin extract.

Claim 43 (original): The method of claim 42, wherein said *Vaccinium* plant material is from *Vaccinium macrocarpon*.

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Claim 44 (original): The method of claim 42, wherein said plant material is from leaves, mature fruit, immature fruit, stems or roots.

Claim 45 (original): The method of claim 43, wherein said plant material is from leaves.

Claim 46 (original): The method of claim 42, wherein said aqueous extraction solvent comprises about 40% acetone, about 40% methanol and about 0.1% ascorbic acid.

Claim 47 (original): The method of claim 43, wherein said aqueous extraction solvent comprises about 40% acetone, about 40% methanol and about 0.1% ascorbic acid.

Claim 48 (original): A proanthocyanidin extract prepared by the method of any one of claims 20-30, 32-40 or 42-47.

Claim 49 (original): A proanthocyanidin extract prepared by the method of claim 31.

Claim 50 (original): A proanthocyanidin extract prepared by the method of claim 41.

Claim 51 (previously presented): A pharmaceutical composition comprising the proanthocyanidin extract of any one of claims 1 to 13 and a pharmaceutically acceptable carrier.

Claim 52 (canceled).

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Claim 53 (original): A pharmaceutical composition comprising the proanthocyanidin extract of claim 48 and a pharmaceutically acceptable carrier.

Claim 54 (original): A pharmaceutical composition comprising the proanthocyanidin extract of claim 49 and a pharmaceutically acceptable carrier.

Claim 55 (original): A pharmaceutical composition comprising the proanthocyanidin extract of claim 50 and a pharmaceutically acceptable carrier.

Claim 56 (previously presented): A method of preventing or treating a urogenital infection in a mammal which comprises administering a pharmaceutical composition to said mammal in an amount and for a time sufficient to prevent, reduce or eliminate symptoms associated with said infection, wherein said pharmaceutical composition comprises a pharmaceutically-acceptable carrier in admixture with one or more

(a) substantially purified plant proanthocyanidin extracts substantially free of anthocyanins and flavonols and capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*;

(b) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*, wherein said polymer comprises two or more flavanoid monomer units wherein at least two of said units are linked together by an A-type interflavanoid linkage by bonds between C4 and C8 and between the C2 and the oxygen of C7

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of the units and the remainder of any units are linked to each other by a B-type interflavanoid bond between C4 and C8 or between C4 and C6 of the units; or

(c) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*.

Claim 57 (original): The method of claim 56, wherein said mammal is a cat or a dog.

Claim 58 (original): The method of claim 56, wherein said mammal is a human.

Claim 59 (original): The method of claim 56, wherein said urogenital infection is a bladder infection or a kidney infection.

Claim 60 (original): The method of claim 59, wherein said kidney infection is pyelonephritis.

Claim 61 (previously presented): A food composition comprising a consumable carrier in admixture with one or more

(a) substantially purified plant proanthocyanidin extracts substantially free of anthocyanins and flavonols and capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*;

(b) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*, wherein said polymer comprises

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two or more flavanoid monomer units wherein at least two of said units are linked together by an A-type interflavanoid linkage by bonds between C4 and C8 and between the C2 and the oxygen of C7 of the units and the remainder of any units are linked to each other by a B-type interflavanoid bond between C4 and C8 or between C4 and C6 of the units; or

(c) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*.

Claim 62 (original): The food composition of claim 61 wherein said consumable carrier is livestock feed.

Claim 63 (original): A method of preventing or treating a urogenital infection in a livestock animal which comprises administering the food composition of claim 62 to said animal in an amount and for a time to prevent, reduce or eliminate symptoms associated with said infection.

Claim 64 (original): A method of reducing the pathogenicity of P-type *E. coli* in the digestive tracts of an animal which comprises administering the food composition of claim 62 to said cattle for a time and in an amount to reduce the detectable number of P-type *E. coli* bacterial cells in the feces or urine of said animal.

Claim 65 (original): The method of claim 64, wherein said animal is a cow, a steer, a calf, a pig, a lamb, a chicken or a turkey.

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Claim 66 (previously presented): A method of reducing P-type *E. coli* contamination in ground meat which comprises:

- (a) obtaining raw meat from an animal;
- (b) adding the food composition of claim 61 to said raw meat; and
- (c) preparing ground meat from said raw meat.

Claim 67 (original): The method of claim 66 wherein said composition is added to said raw meat before or during preparation of said ground meat.

Claim 68 (previously presented): A method of reducing P-type *E. coli* contamination in ground meat which comprises:

- (a) obtaining raw meat from an animal;
- (b) adding to said raw meat one or more
 - (i) substantially purified plant proanthocyanidin extracts substantially free of anthocyanins and flavonols and capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*;
 - (ii) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*, wherein said polymer comprises two or more flavanoid monomer units wherein at least two of said units are linked together by an A-type interflavanoid linkage by bonds between C4 and C8 and between the C2 and the oxygen of C7 of the units and the remainder of any units are linked to each other by a B-type interflavanoid bond between C4 and C8 or between C4 and C6 of the units; or

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(iii) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*; and

(c) preparing ground meat from said raw meat.

Claim 69 (original): The method of claim 68 wherein said composition is added to said raw meat before or during preparation of said ground meat.

Claim 70 (original): A method of reducing P-type *E. coli* contamination in ground meat which comprises:

(a) feeding the food composition of claim 62 to a livestock animal;

(b) obtaining raw meat from said animal; and

(c) preparing a ground meat from said raw meat.

Claim 71 (original): The method of claim 70, wherein said ground meat is prepared using a proportion of said raw meat sufficient, when detected by an agglutination assay, to reduce the agglutination of *E. coli* microorganisms in said ground meat relative to ground meat prepared only from raw meat of a similar livestock animal who has not been fed said feed composition.

Claim 72 (original): The food composition of claim 61 wherein said consumable carrier is domestic animal feed.

Claim 73 (original): A method of preventing or treating a urogenital infection in a domesticated animal which comprises administering the food composition of claim 72 to said animal in

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an amount and for a time to prevent, reduce or eliminate symptoms associated with said infection.

Claim 74 (original): The method of claim 73, wherein said animal is a cat or a dog.

Claim 75 (original): The food composition of claim 61 wherein said consumable carrier is a consumable food product.

Claim 76 (original): The composition of claim 75, wherein said consumable food product is a cranberry-containing food product.

Claim 77 (original): The composition of claim 76, wherein said cranberry-containing food product is a dried cranberry, a sweetened and dried cranberry, a flavored fruit piece, a sauce, a jelly, a relish, juice, wine or a cranberry juice-containing product.

Claim 78 (original): The composition of claim 75, wherein said consumable food product is a beverage.

Claim 79 (original): The composition of claim 78, wherein said beverage comprises cranberry juice, unpasteurized juice or pasteurized juice.

Claim 80 (original): The food composition of claim 75 wherein said consumable food product is ground meat.

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Claim 81 (original): A method of preventing or treating a urogenital infection in a human which comprises administering a food composition of any one of claims 75-80 to said human in an amount and for a time to prevent, reduce or eliminate symptoms associated with said infection.

Claim 82 (previously presented): A method of preventing or treating diarrhea in a mammal which comprises administering a pharmaceutical composition to said mammal in an amount and for a time sufficient to prevent, reduce or eliminate symptoms associated with said diarrhea, wherein said pharmaceutical composition comprises a pharmaceutically-acceptable carrier in admixture with one or more

(a) substantially purified plant proanthocyanidin extracts substantially free of anthocyanins and flavonols and capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*;

(b) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*, wherein said polymer comprises two or more flavanoid monomer units wherein at least two of said units are linked together by an A-type interflavanoid linkage by bonds between C4 and C8 and between the C2 and the oxygen of C7 of the units and the remainder of any units are linked to each other by a B-type interflavanoid bond between C4 and C8 or between C4 and C6 of the units; or

(c) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*.

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Claim 83 (original): The method of claim 82, wherein said mammal is a cat or a dog.

Claim 84 (original): The method of claim 82, wherein said mammal is a human.

Claim 85 (previously presented): A method of inhibiting adherence of P-type *E. coli* to a surface which comprises contacting said bacteria with at least one proanthocyanidin extract or polymer prior to or concurrently with contacting said bacteria with said surface, wherein said proanthocyanidin extract or polymer is selected from the group consisting of

(a) a substantially purified plant proanthocyanidin extract substantially free of anthocyanins and flavonols and capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*;

(b) a proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*, wherein said polymer comprises two or more flavanoid monomer units wherein at least two of said units are linked together by an A-type interflavanoid linkage by bonds between C4 and C8 and between the C2 and the oxygen of C7 of the units and the remainder of any units are linked to each other by a B-type interflavanoid bond between C4 and C8 or between C4 and C6 of the units; or

(c) a proanthocyanidin polymer capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*.

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Claim 86 (original): The method of claim 85, wherein said surface is a uroepithelial cell surface or biofilm.

Claim 87 (previously presented): A method of reducing the incidence of infection after surgery, treating topical wounds or acne, or preventing or eliminating oral infection which comprises administering a pharmaceutical composition to a site of infection or potential infection in a patient, wherein said pharmaceutical composition comprises a pharmaceutically-acceptable carrier in admixture with one or more

(a) substantially purified plant proanthocyanidin extracts substantially free of anthocyanins and flavonols and capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*;

(b) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*, wherein said polymer comprises two or more flavanoid monomer units wherein at least two of said units are linked together by an A-type interflavanoid linkage by bonds between C4 and C8 and between the C2 and the oxygen of C7 of the units and the remainder of any units are linked to each other by a B-type interflavanoid bond between C4 and C8 or between C4 and C6 of the units; or

(c) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*.

Claim 88 (previously presented): A method of detecting P-type reactive bacteria in a body fluid sample which comprises

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(a) contacting said body fluid sample with a P-type receptor-specific assay reagent and for a time and in an amount to allow binding of any P-type reactive bacteria present in said sample to said reagent, wherein said reagent comprises a solid-phase substrate coated with one or more proanthocyanidin extracts, compounds or polymers selected from the group consisting of

(i) substantially purified plant proanthocyanidin extracts substantially free of anthocyanins and flavonols and capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*;

(ii) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*, wherein said polymer comprises two or more flavanoid monomer units wherein at least two of said units are linked together by an A-type interflavanoid linkage by bonds between C4 and C8 and between the C2 and the oxygen of C7 of the units and the remainder of any units are linked to each other by a B-type interflavanoid bond between C4 and C8 or between C4 and C6 of the units; or

(iii) proanthocyanidin polymers capable of inhibiting agglutination of P-type *E. coli* but incapable of inhibiting agglutination of type 1 *E. coli*; and

(b) determining whether P-type reactive bacteria are present in said sample by assessing the degree of agglutination in said sample.

Claim 89 (original): The method of claim 88, wherein said plant extract is from a *Vaccinium* species.

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Claim 90 (previously presented): The method of claim 89,
wherein said *Vaccinium* species is *Vaccinium macrocarpon*.